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SHV-Type Extended-Spectrum Beta-Lactamase Production Is Associated with Reduced Cefepime Susceptibility in *Enterobacter cloacae*

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Cefepime is a potentially useful antibiotic for treatment of infections with *Enterobacter cloacae*. However, in our institution the MIC_{90} for *E. cloacae* bloodstream isolates is 16 μg/ml. PCR amplification of *bla* genes revealed that one-third (15/45) of *E. cloacae* bloodstream isolates produced SHV-type extended-spectrum beta-lactamases (ESBLs) in addition to hyperproduction of AmpC-type beta-lactamases. The majority (11/15) of ESBL producers also produced the TEM-1 beta-lactamase. The SHV types included SHV-2, -5, -7, -12, -14, and -30. All but two of the ESBL-producing *E. cloacae* isolates, but none of the non-ESBL-producing strains, had MICs of cefepime of ≥2 μg/ml. The MIC_{90} for cefepime for ESBL-producing strains was 64 μg/ml, while for non-ESBL producers it was 0.5 μg/ml. Using current Clinical and Laboratory Standards Institute breakpoints for cefepime, two thirds (10/15) of ESBL-producing isolates would have been regarded as susceptible to cefepime. Phenotypic ESBL detection methods were generally unreliable with these *E. cloacae* isolates. Based on these results, pharmacokinetic, pharmacodynamic, and clinical reevaluation of cefepime breakpoints for *E. cloacae* may be prudent.

Enterobacter cloacae is a leading cause of ventilator-associated pneumonia, bloodstream infections, and urinary tract infections in hospitalized patients. A chromosomal gene in *E. cloacae* characteristically encodes the AmpC beta-lactamase (9). Mutations that increase the expression of the gene encoding AmpC are responsible for the emergence of resistance of the organism to cephalosporins such as ceftriaxone, cefotaxime, and ceftaxidime during therapy (4, 11). Approximately 30% of *E. cloacae* isolates from patients in intensive care units in the United States are resistant to cephalosporins such as ceftriaxone, cefotaxime, and ceftaxidime (15).

Cefepime is potentially a very useful antibiotic for the treatment of serious infections with *E. cloacae*, even in the presence of increased production of the AmpC beta-lactamase (23). In vitro studies performed prior to the commercial availability of cefepime showed that the MIC_{90} ranged from 0.06 to 0.5 μg/ml (12, 25, 26). In contrast, an assessment of isolates from North American intensive care units in 2003 showed that the MIC_{90} was 2 μg/ml, with MICs of some isolates as high as >16 μg/ml (20). In our institution in 2003, the MIC_{90} of cefepime for *E. cloacae* bloodstream isolates was 16 μg/ml. For this reason, we sought to investigate the mechanisms of reduced cefepime susceptibility in bloodstream isolates occurring at our institution.

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MATERIALS AND METHODS

Bacterial strains. Consecutive bloodstream isolates of *E. cloacae* from patients at the University of Pittsburgh Medical Center (UPMC) were studied. The isolates were collected from March 2003 through July 2004. Species identification was done by standard biochemical tests. The strains were supplied by the hospital's clinical microbiology laboratory as part of an Institutional Review Board approved study on mechanisms of antibiotic resistance in hospital pathogens.

Antibiotic susceptibility. The clinical microbiology laboratory at UPMC routinely assesses the MICs of cefepime and other injectable antibiotics commonly used in the treatment of infections with gram-negative bacteria by broth microdilution methods using Clinical and Laboratory Standards Institute (CLSI) criteria, current as of 1 January 2005. Additionally, the MICs of piperacillin-tazobactam, cefotaxime, ceftazidime, ceftazidime-clavulanic acid, cefotaxime, cefotaxime-clavulanic acid, cefepime, cefepime-clavulanic acid, cefoxitin, gentamicin, ertapenem, imipenem, meropenem, and ciprofloxacin were determined by Etest for the strains (AB Biodisk, Solna, Sweden). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the reference strains for antimicrobial susceptibility testing.

Analytical isoelectric focusing. Analytical isoelectric focusing was performed on isolates with cefepime MICs of 0.5 μg/ml or more, as described previously (19). After removal of whole cells and debris by centrifugation, the supernatant was used to determine the isoelectric point (pI). Electrophoresis was performed using precast polyacrylamide gels, pH 3 to 10 (Bio-Rad, Hercules, CA). Enzyme activity was detected by placing filter paper soaked in nitrocefin (500 μg/ml) (Becton Dickinson, Sparks, MD) over the focused gel. Standards from Bio-Rad (Bio-Rad, Hercules, CA) were used, with the following isoelectric points: 4.45, 4.65, 4.75, 5.1, 6.0, 6.5, 6.8, 7.0, 7.1, 7.5, 7.8, 8.00, 8.20, and 9.6.

Plasmid profiles. The plasmid DNA of the extended-spectrum beta-lactamase (ESBL)-producing clinical isolates was extracted with a plasmid extraction kit (Wizard Plus Minipreps DNA purification system; Promega, Madison, WI), according to the manufacturer's instructions. Plasmid DNA electrophoresis was performed with 0.8% agarose gel and visualized with ethidium bromide under UV light. λ HindIII (Promega, Madison, WI) was used as a molecular size marker.

PFGE. Genomic DNA was isolated and digested with XbaI (New England Biolabs, Beverly, Mass.). Pulsed-field gel electrophoresis (PFGE) was performed with the CHEF III system (Bio-Rad, Hercules, CA) with the following run parameters: block I, with a switch time of 3 to 65 s and a run time of 17 h, and block II, with a switch time of 15 to 30 s and a run time of 6 h. Dendrograms were created with BioNumerics (Bio-Rad, Hercules CA) by using the Dice coefficient, unweighted-pair group method with arithmetic means, and a position tolerance of 1.3%. Relatedness of the isolates was determined by the criteria of Tenover et al. (24).

Detection of ESBL genes by PCR. Detection of genes encoding ESBLs was attempted for all isolates with cefepime MICs of 0.5 μg/ml or more. A single colony of each test isolate was resuspended in 400 μl water and boiled for 15 min. The resulting supernatant was used as a bacterial template DNA in PCR assays. The primers for detection of the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes are as follows: 5′-ATGAGTATTCAACATTTCCGTG-3′ and 5′-TTACCAATGCTTAATCAGTGAG-3′ for bla_{TEM} (6), 5′-ATTTGTCGCTTCTTTACTCGC-3′ and 5′-TTTATGGCGTTACCTTTGACC-3′ for bla_{SHV} (6), and 5′-CGCTTTGCGATGTGCAG-3′ and 5′-ACCGCGATATCGTTGGT-3′ for bla_{CTX-M} (2). PCRs were performed with Red*Taq* DNA polymerase (Sigma, St Louis, MO), according to the instructions of the manufacturer, in the presence of 2 μl of the template DNA preparation in a total volume of 30 μl. The DNA amplification programs consisted of an initial denaturation step (96°C, 5 min) followed by 30 cycles of denaturation (96°C, 30 s), annealing (annealing temperature designed for each primer set, 30 s), and extension (72°C, 1 min), and a final extension of 5 min at 72°C. Ten microliters of reaction mixture containing the PCR product was analyzed by electrophoresis in 0.8% (wt/vol) agarose (Bio-Rad, Hercules, CA).

Sequence analysis. The amplified products were sequenced using ABI 4500 and ABI 3100 genetic analyzers according to the manufacturer's instructions. Sequencing reactions were performed with corresponding primers specific for the bla_{TEM-1} and bla_{SHV-1} genes used for the previous amplification. Sequence analysis was performed using Lasergene DNASTAR sequencing analysis software (DNAStar, Madison, WI). Each sequence of bla_{TEM} and bla_{SHV} genes was identified by comparison with known ESBL sequences available in the GenBank and EMBL databases by multiple-sequence alignment using the BLAST program.

Phenotypic detection of beta-lactamase production. Disk diffusion testing was performed using ceftazidime, cefotaxime, and cefepime alone (30 μg each; Remel, Lenexa, KS), and in combination with clavulanic acid: ceftazidime-clavulanic acid, cefotaxime-clavulanic acid (30/10 μg each; Becton Dickinson, Sparks, MD), and cefepime-clavulanic acid (30/10 μg; Rosco, Prolab) disks were used. The disk tests were performed using confluent growth on Mueller-Hinton agar (Becton Dickinson, Sparks, MD), incubated at 35°C for 24 h. The sensitivities and specificities of phenotypic confirmatory tests for ESBL detection in *Klebsiella* spp. and *E. coli*, but applied to *E. cloacae*, were determined. No interpretative criteria for use of cefepime versus cefepime-clavulanic acid exist for any species.

Additionally, double-disk synergy tests were performed by placing disks of ceftazidime, cefotaxime, and cefepime at distances of 20 and 30 mm (center to center) from a disk containing amoxicillin plus clavulanic acid (20/10 μg; Remel, Lenexa, KS). A "keyhole" phenomenon was regarded as positive for ESBL production.

MICs for cefotaxime, ceftazidime, and cefepime were determined by using Etest strips. These MICs were compared to those obtained from the same isolates by using Etest strips containing ceftazidime-clavulanic acid, cefotaxime-clavulanic acid, and cefepime-clavulanic acid.

Classification of *E. cloacae* strains as having inducible, partially derepressed, or derepressed AmpC production was determined by the methods of Sanders et al. (22), in which the cefoxitin-cefotaxime antagonist test was performed (16, 22). The *E. cloacae* isolates were categorized as follows: derepressed AmpC mutants had a cefoxitin MIC of ≥32 μg/ml, a cefotaxime MIC of ≥16 μg/ml, and a negative cefoxitin-cefotaxime antagonist test; partially derepressed AmpC mutants had the same characteristics as derepressed AmpC mutants but with a positive cefoxitin-cefotaxime antagonist test; and inducible AmpC-producing strains had a cefotaxime MIC of ≤8 μg/ml and a positive cefoxitin-cefotaxime antagonist test (16).

RESULTS

PFGE. There was no relatedness between 41/45 isolates. All 30 isolates which were found not to be ESBL producers were unrelated. Two of 15 ESBL producers (ES1 and ES22) were closely related, and two additional ESBL-producing strains (ES31 and ES43) were possibly related (data not shown).

TABLE 1. Antibiotic susceptibilities (μg/ml) of *E. cloacae* strains without ESBL production

Antibiotic(s)	Susceptibility (μg/ml) of[a]:		
	Inducible strains	Partially derepressed strains	Derepressed mutants
Piperacillin-tazobactam	0.75–3	0.38–8	16–>256
Piperacillin	0.5–64	0.5–24	32–>256
Cefoxitin	0.19–6	12–128	128–>256
Ceftazidime	0.125–0.5	0.094–12	12–>256
Ceftazidime-clavulanic acid	0.38–>4	0.064–1.5	>4
Cefotaxime	0.045–1	0.125–12	24–128
Cefotaxime-clavulanic acid	0.125–1	0.125–>1	>1
Cefepime	0.032–0.094	0.064–0.5	0.125–0.75
Cefepime-clavulanic acid	0.064–0.094	0.016–0.5	0.016–0.5
Ciprofloxacin	0.006–0.012	0.006–0.094	0.016–>32
Imipenem	0.19–0.25	0.19–0.5	0.25–0.38
Gentamicin	0.25–0.5	0.094–0.75	0.25–1

[a] Results are shown for 4 inducible strains, 20 partially derepressed strains, and 6 derepressed mutants.

Antibiotic susceptibility. The antibiotic susceptibilities of the non-ESBL- and ESBL-producing *E. cloacae* strains are seen in Tables 1 and 2, respectively. Forty percent (18/45) of isolates had cefepime MICs of 0.5 μg/ml or more. The distribution of the cefepime MICs is shown in Fig. 1. The MIC_{90} for cefepime for ESBL-producing strains was 64 μg/ml, while for non-ESBL producers it was 0.5 μg/ml.

Analytical isoelectric focusing and genotypic detection of ESBLs. Fifteen of 18 isolates with a cefepime MIC of 0.5 μg/ml or more carried ESBL genes. All of the ESBLs were of the SHV type (Table 3). Eleven of the 15 ESBL-producing isolates harbored bla_{TEM-1} in addition to bla_{SHV}. bla_{CTX-M} genes were not amplified from any isolates by using our primers. The ESBLs encoded by the *E. cloacae* strains included SHV-2 (one isolate), SHV-5 (one isolate), SHV-7 (eight isolates), SHV-12 (two isolates), SHV-14 (three isolates), and SHV-30 (one isolate). One isolate produced both SHV-7 and SHV-30. The isoelectric points of the isolates are presented in Table 3. The three non-ESBL producers with cefepime MICs of 0.5 μg/ml or more produced only one beta-lactamase each, with an isoelectric point consistent with that for AmpC.

Plasmid profile analysis. Selected ESBL-producing *E. cloacae* strains isolated during this period were analyzed for their plasmid content (Fig. 2; Table 3). All ESBL-producing *E. cloacae* isolates harbored plasmids, although there was diversity in the sizes of these plasmids (Fig. 2).

Antibiotic susceptibility testing of ESBL-producing strains. Antibiotic susceptibility testing results are shown in Table 2. The cefepime MIC distributions of ESBL-producing isolates were 0.5 μg/ml (one isolate), 1 μg/ml (one isolate), 2 μg/ml (three isolates), 4 μg/ml (two isolates), 6 μg/ml (one isolate), 8 μg/ml (two isolates), 12 μg/ml (one isolate), 16 μg/ml (two isolates), 64 μg/ml (one isolate), and >256 μg/ml (one isolate). According to current CLSI breakpoints for *Enterobacteriaceae*, 10/15 isolates would have been regarded as cefepime susceptible, 3/15 as cefepime intermediate, and 2/15 as cefepime resistant. The majority of ESBL-producing isolates were non-

TABLE 2. MIC values of the ESBL-producing *E. cloacae* isolates

Isolate	MIC (μg/ml) for:											
	Piperacillin-tazobactam	Ceftazidime	Ceftazidime-clavulanic acid	Cefotaxime	Cefotaxime-clavulanic acid	Cefepime	Cefepime-clavulanic acid	Ciprofloxacin	Ertapenem	Imipenem	Meropenem	Gentamicin
ES1	16	>256	>4	128	>1	4	0.125	0.38	0.125	0.25	0.032	24
ES6	>256	>256	>4	>256	>1	8	>4	>32	2	0.25	0.19	256
ES7	>256	>256	>4	>256	>1	>256	>4	0.064	0.75	0.19	0.064	0.75
ES11	8	48	>4	16	>1	2	0.5	0.5	3	0.19	0.125	8
ES15	>256	>256	>4	>256	>1	6	4	0.012	0.75	0.25	0.094	4
ES18	>256	>256	>4	>256	>1	64	>4	8	4	0.38	0.38	48
ES20	>256	>256	>4	>256	>1	8	>4	0.125	2	0.25	0.094	4
ES22	8	256	>4	192	>1	2	0.094	0.38	0.5	0.5	0.032	128
ES24	>256	>256	>4	12	>1	16	2	>32	2	0.125	0.19	>256
ES31	1.5	128	>4	8	>1	1	<0.016	0.094	0.125	0.25	0.032	256
ES37	>256	>256	>4	>256	>1	12	>4	8	2	0.25	0.19	48
ES40	12	>256	>4	96	>1	4	1.5	8	1	0.38	0.064	96
ES43	8	>256	1	64	>1	2	0.38	4	0.125	0.125	0.016	96
ES44	>256	>256	>4	>256	>1	16	>4	4	1	0.25	0.125	64
ES45	16	2	0.5	1	>1	0.5	0.19	6	0.38	0.125	0.032	8

susceptible to numerous antibiotics: 14/15 ESBL-producing isolates were nonsusceptible to ceftazidime, 8/15 were nonsusceptible to piperacillin-tazobactam, 12/15 were nonsusceptible to gentamicin, 8/15 were nonsusceptible to ciprofloxacin, and 2/15 were nonsusceptible to ertapenem. All 15 isolates were susceptible to imipenem and meropenem.

Phenotypic detection of ESBL production. Applying CLSI ESBL screening disk diffusion criteria to *E. cloacae* showed that 23 isolates had a "positive" result, but 9 of these isolates were non-ESBL producers (Table 4). Confirmatory results were positive for 7/15 ESBL-producing strains but for no non-ESBL-producing isolate. Applying CLSI ESBL screening MIC criteria gave similar results (Table 5), although confirmatory tests were positive for just 2/15 ESBL-producing strains. The conventional double disk diffusion tests utilizing ceftazidime and cefotaxime disks were rarely positive (Table 4).

Use of cefepime susceptibility results as a marker for ESBL production appeared more useful (Table 6). All 13 isolates with cefepime MICs of 2 μg/ml or more were ESBL producers, and 15/18 (83%) of isolates with cefepime MICs of >0.25 μg/ml were ESBL producers. A confirmatory test with cefepime-clavulanic acid was only 73% sensitive using disk diffusion and 53% sensitive using ≥3 MIC reductions (Table 6).

DISCUSSION

There are now numerous reports of *E. cloacae*-producing ESBLs (1, 3, 7, 8, 10, 14, 16, 17), including several from the

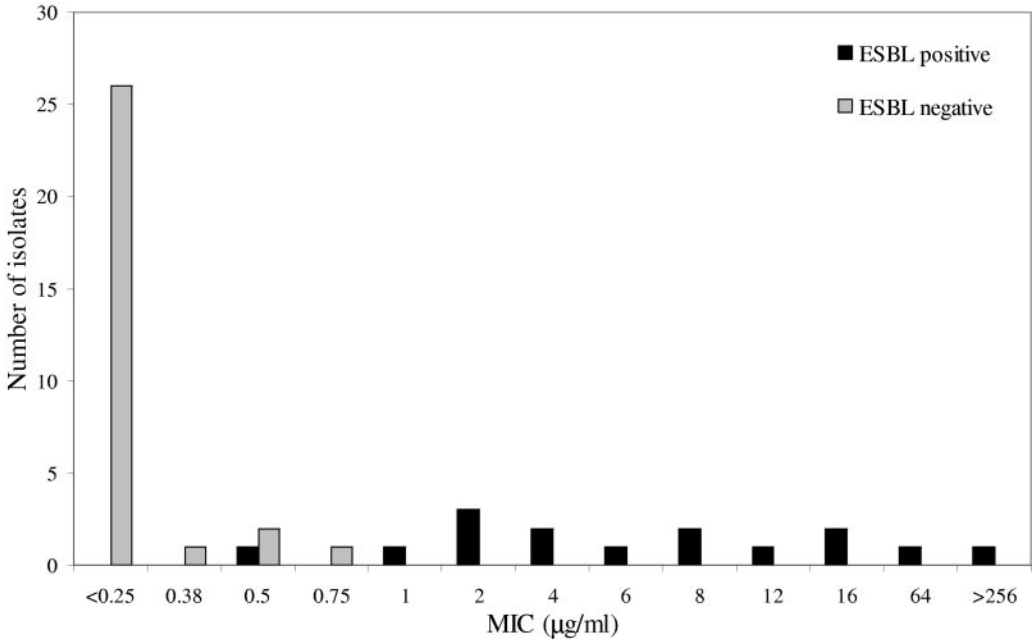

FIG. 1. Distribution of cefepime MICs for *E. cloacae* strains with and without ESBL.

TABLE 3. Characterization of the ESBL-producing *E. cloacae* isolates

Isolate	Beta-lactamase characterization	Genotypic evaluation		pI	Plasmid profile
		SHV	TEM-1		
ES1	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P1
ES6	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P2
ES7	Derepressed AmpC + ESBL	SHV-5	Absent	8.2+9.0	P3
ES11	Derepressed AmpC + ESBL	SHV-14	Present	5.4+7.0+9.0	P4
ES15	Derepressed AmpC + ESBL	SHV-14	Absent	7.0+9.0	P5
ES18	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P6
ES20	Derepressed AmpC + ESBL	SHV-2	Absent	7.6+9.0	P7
ES22	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P1
ES24	Derepressed AmpC + ESBL	SHV-7, SHV-30	Present	5.4+7.0+7.6+9.0	P8
ES31	Derepressed AmpC + ESBL	SHV-12	Present	5.4+8.2+9.0	P9
ES37	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P10
ES40	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P11
ES43	Partially derepressed + ESBL	SHV-12	Present	5.4+8.2+9.0	P9
ES44	Derepressed AmpC + ESBL	SHV-7	Present	5.4+7.6+9.0	P10
ES45	Derepressed AmpC + ESBL	SHV-14	Absent	7.0+9.0	P12

United States (5, 13, 21). The earliest reports of ESBL-producing *E. cloacae* strains from the United States were of TEM-12 and TEM-26 producers from Boston in 1988 (21) and SHV-3 producers from Boston in the 1990s (5). In a subsequent report, Levison and colleagues found SHV-7- and SHV-12-producing *E. cloacae* in three different hospitals in Philadelphia in 2000 and 2001 (13). In Pittsburgh, we have subsequently found SHV-2, -5, -7, -12, -14, and -30 in *E. cloacae* strains. In a 1-year period, 33% (15/45) of *E. cloacae* bloodstream isolates were found to be ESBL producers. Un-

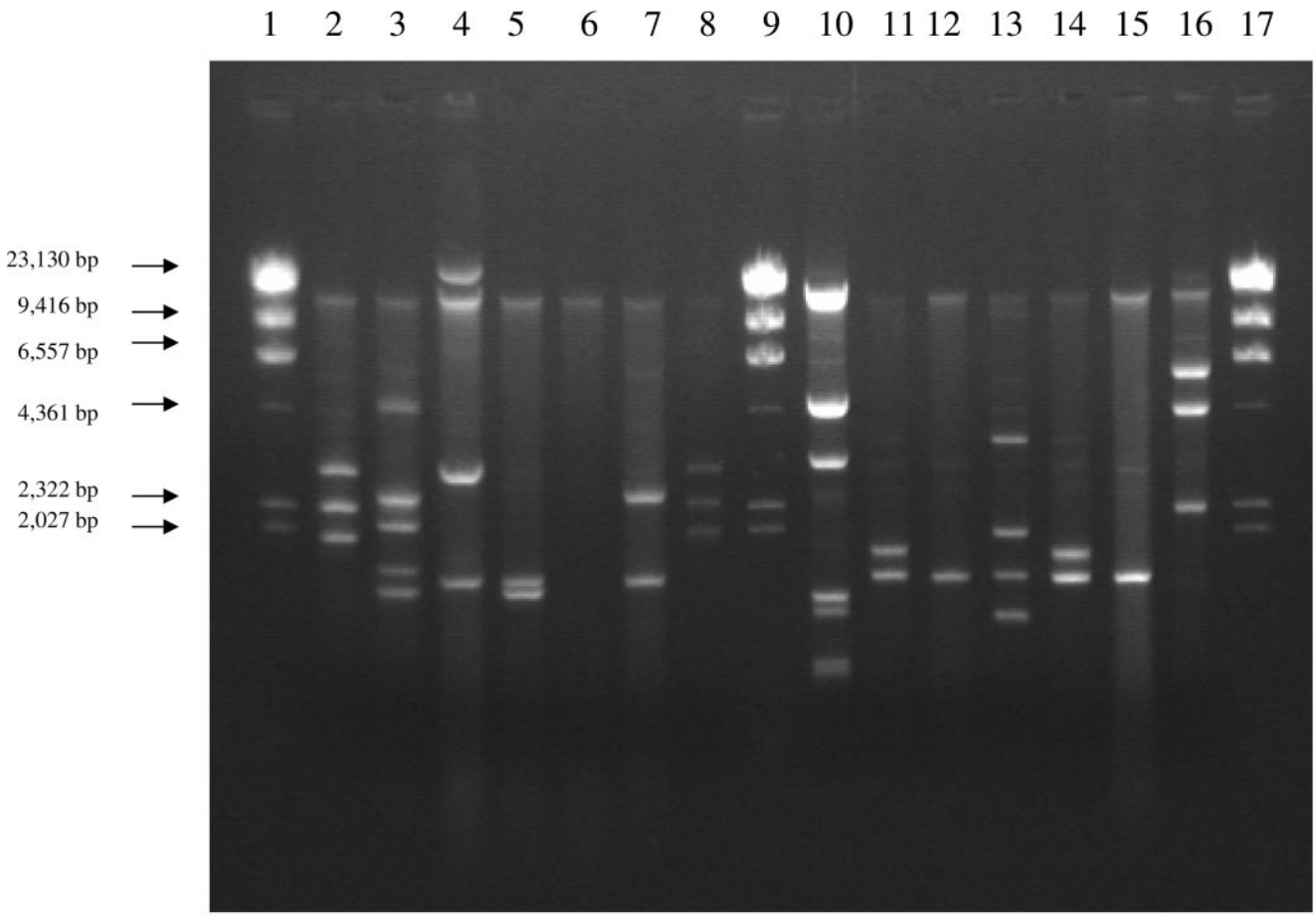

FIG. 2. Plasmids from representative ESBL-producing *E. cloacae* strains. Lanes 1, 9, and 17 contain λ HindIII size markers. Lanes 2 to 8 contain ESBL-producing *E. cloacae* strains ES1, ES6, ES7, ES11, ES15, ES18, and ES22, respectively. Lanes 10 to 16 contain *E. cloacae* strains ES24, ES31, ES37, ES40, ES43, ES44, and ES45, respectively.

TABLE 4. Distribution of the results of different disk diffusion techniques

Test and drug	Test result	Category (no. of isolates)		Sensitivity	Specificity
		ESBL	Non-ESBL		
CLSI ESBL initial screen[a]					
Ceftazidime	Positive	14	9	0.93	0.70
	Negative	1	21		
Cefotaxime	Positive	14	9	0.93	0.70
	Negative	1	21		
CLSI ESBL phenotypic confirmatory test[b]					
Ceftazidime	Positive	7	0	0.47	1
	Negative	8	30		
Cefotaxime	Positive	5	0	0.33	1
	Negative	10	30		
Double disk diffusion test (20 mm)					
Ceftazidime	Positive	6	0	0.40	1
	Negative	9	30		
Cefotaxime	Positive	7	0	0.47	1
	Negative	8	30		
Double disk diffusion test (30 mm)					
Ceftazidime	Positive	1	0	0.07	1
	Negative	14	30		
Cefotaxime	Positive	7	0	0.47	1
	Negative	8	30		

[a] The CLSI disk inhibition break points for ESBL initial screen of *E. coli* and *Klebsiella* spp. were <22 mm for ceftazidime (30 μg) and <27 mm for cefotaxime (30 μg).
[b] A >5-mm increase in zone diameter in combination with clavulanic acid versus when tested alone.

fortunately, there are no surveillance data from the United States in order to determine whether this percentage is high or whether it is within the range found in similar hospitals. In a recently published study from Korea, 43% of *E. cloacae* blood culture isolates were found to be ESBL producers (16).

Wild-type *E. cloacae* strains obtained prior to the commercial release of cefepime had cefepime MICs of 0.06 to 0.5 μg/ml. It is uncertain whether any of the strains tested in these studies produced ESBLs. In our center, the MIC_{90} for cefepime for ESBL-producing strains was 64 μg/ml, while for non-ESBL producers it was 0.5 μg/ml. Studies examining outer membrane proteins, efflux pumps, and the expression levels and hydrolytic activities of SHV-type ESBLs against cefepime are currently under way. Thus, at the present time, we are unable to conclusively state that the production of ESBLs is responsible for the elevated cefepime MICs that we observed.

TABLE 5. Distribution of the results of MICs obtained by Etest as a means of ESBL detection and phenotypic confirmation

Test stage and drug	Test result	Category (no. of isolates)		Sensitivity	Specificity
		ESBL	Non-ESBL		
Initial screening[a]					
Ceftazidime	Positive	15	8	1	0.73
	Negative	0	22		
Cefotaxime	Positive	15	8	1	0.73
	Negative	0	22		
Phenotypic confirmation[b]					
Ceftazidime	Positive	2	0	0.13	1
	Negative	13	30		
Cefotaxime	Positive	0	0	0	1
	Negative	15	30		

[a] The CLSI MIC breakpoints for ESBL screening for *E. coli* and *Klebsiella* spp. were 2 μg/ml or more for ceftazidime and cefotaxime.
[b] A >3 twofold-concentration decrease in MIC for either antimicrobial agent tested in combination with clavulanic acid versus its MIC tested alone.

The clinical implications of this elevation in cefepime MIC are under evaluation, since some patients in our series failed cefepime therapy. Assessments of the pharmacokinetics and pharmacodynamics of cefepime presented at CLSI Antimicrobial Susceptibility Testing subcommittee meetings would suggest that cefepime may not be effective for treating serious infections when dosed at 1 g every 12 h for an organism with a MIC of 8 μg/ml or higher. Clinical data from patients with serious infections with ESBL-producing *Klebsiella* spp. or *E. coli* would support the concept that cefepime activity may be compromised against some ESBL-producing organisms with MICs in the current susceptible range (18, 27). Thus, it would appear prudent for cefepime breakpoints to be reconsidered.

Given that we have evaluated just 15 ESBL-producing isolates and 30 non-ESBL-producing isolates, we cannot categorically comment on ESBL detection methods for *E. cloacae*. Conventional methods using cefotaxime or ceftazidime are likely to be unreliable. However, a cefepime MIC of ≥2 μg/ml appears to be a consistently robust marker of ESBL production. While tests incorporating cefepime plus clavulanic acid may detect some ESBL producers with lower MICs, the tests were not more than 75% sensitive in our hands.

Why would SHV-type ESBLs evolve in an *E. cloacae* host? In our isolates, the SHV-type ESBLs are present in the company of a derepressed AmpC. Increased production of the AmpC beta-lactamase confers resistance to oxyiminocephalos-

TABLE 6. Distribution of the results using cefepime as an indicator for ESBL production

Test and result	Category (no. of isolates)		Sensitivity	Specificity
	ESBL	Non-ESBL		
Disk diffusion screening test[a]				
Positive	13	0	0.87	1
Negative	2	30		
Disk diffusion confirmatory test[b]				
Positive	11	0	0.73	1
Negative	4	30		
Double disk diffusion test (20 mm)				
Positive	13	0	0.87	1
Negative	2	30		
Double disk diffusion test (30 mm)				
Positive	8	0	0.53	1
Negative	7	30		
MIC-based screening test[c]				
Positive	14	0	0.93	1
Negative	1	30		
MIC-based confirmatory test[d]				
Positive	8	0	0.53	1
Negative	7	30		

[a] Based on zone diameter of <27 mm as a screen for potential ESBL production.
[b] A >5-mm increase in zone diameter of cefepime in combination with clavulanic acid versus cefepime tested alone.
[c] Cefepime breakpoint based on our study was 1 μg/ml.
[d] A >3 twofold-concentration decrease in MIC for either antimicrobial agent tested in combination with clavulanic acid versus its MIC tested alone.

porins and beta-lactam/beta-lactamase inhibitor combinations. The presence of an ESBL adds a selective advantage against cefepime. Alternatively, by production of large quantities of AmpC, protection against cephalosporins and beta-lactamase inhibitors may create a permissive microbiological environment within which ESBLs may evolve. It is known that ESBLs are not catalytically efficient against penicillins and are hypersusceptible to beta-lactamase inhibitors. This trade-off in catalytic activity would be compensated by a robust penicillinase, TEM-1, and a cephalosporinase that is inhibitor resistant. The net result would be expansion of resistance to cefepime.

In summary, in our center, ESBL production by *E. cloacae* isolates causing serious infections is common. Thirty-three percent of bloodstream isolates were ESBL producers. Occasionally, patients shared genotypically similar isolates, but most often ESBL-producing isolates appeared to arise de novo. Given clinical concerns regarding the efficacy of cefepime against some ESBL-producing strains, reassessment of cefepime breakpoints by CLSI would seem prudent.

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REFERENCES

1. **Bell, J. M., J. D. Turnidge, R. N. Jones, and the SENTRY Asia-Pacific Participants.** 2003. Prevalence of extended-spectrum β-lactamase-producing *Enterobacter cloacae* in the Asia-Pacific region: results from the SENTRY Antimicrobial Surveillance Program, 1998 to 2001. Antimicrob. Agents Chemother. **47:**3989–3993.
2. **Bonnet, R., J. L. M. Sampaio, R. Labia, C. De Champs, D. Sirot, C. Chanal, and J. Sirot.** 2000. A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob. Agents Chemother. **44:**1936–1942.
3. **Bouchillon, S. K., B. M. Johnson, D. J. Hoban, J. L. Johnson, M. J. Dowzicky, D. H. Wu, M. A. Visalli, and P. A. Bradford.** 2004. Determining incidence of extended spectrum beta-lactamase producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int. J. Antimicrob. Agents **24:**119–124.
4. **Chow, J. W., M. J. Fine, D. M. Shlaes, J. P. Quinn, D. C. Hooper, M. P. Johnson, R. Ramphal, M. M. Wagener, D. K. Miyashiro, and V. L. Yu.** 1991. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann. Intern. Med. **115:**585–590.
5. **D'Agata, E., L. Venkataraman, P. DeGirolami, L. Weigel, M. Samore, and F. Tenover.** 1998. The molecular and clinical epidemiology of enterobacteriaceae-producing extended-spectrum beta-lactamase in a tertiary care hospital. J. Infect. **36:**279–285.
6. **Essack, S. Y., L. M. C. Hall, D. G. Pillay, M. L. McFadyen, and D. M. Livermore.** 2001. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. Antimicrob. Agents Chemother. **45:**88–95.
7. **Giakkoupi, P., L. S. Tzouvelekis, A. Tsakris, V. Loukova, D. Sofianou, and E. Tzelepi.** 2000. IBC-1, a novel integron-associated class A β-lactamase with extended-spectrum properties produced by an *Enterobacter cloacae* clinical strain. Antimicrob. Agents Chemother. **44:**2247–2253.
8. **Ho, P. L., R. H. Shek, K. H. Chow, R. S. Duan, G. C. Mak, E. L. Lai, W. C. Yam, K. W. Tsang, and W. M. Lai.** 2005. Detection and characterization of extended-spectrum beta-lactamases among bloodstream isolates of *Enterobacter* spp. in Hong Kong, 2000–2002. J. Antimicrob. Chemother. **55:**326–332.
9. **Jacoby, G. A., and L. S. Munoz-Price.** 2005. The new beta-lactamases. N. Engl. J. Med. **352:**380–391.
10. **Kartali, G., E. Tzelepi, S. Pournaras, C. Kontopoulou, F. Kontos, D. Sofianou, A. N. Maniatis, and A. Tsakris.** 2002. Outbreak of infections caused by *Enterobacter cloacae* producing the integron-associated β-lactamase IBC-1 in a neonatal intensive care unit of a Greek hospital. Antimicrob. Agents Chemother. **46:**1577–1580.
11. **Kaye, K. S., S. Cosgrove, A. Harris, G. M. Eliopoulos, and Y. Carmeli.** 2001. Risk factors for emergence of resistance to broad-spectrum cephalosporins among *Enterobacter* spp. Antimicrob. Agents Chemother. **45:**2628–2630.
12. **Kessler, R. E., M. Bies, R. E. Buck, D. R. Chisholm, T. A. Pursiano, Y. H. Tsai, M. Misiek, K. E. Price, and F. Leitner.** 1985. Comparison of a new cephalosporin, BMY 28142, with other broad-spectrum β-lactam antibiotics. Antimicrob. Agents Chemother. **27:**207–216.
13. **Levison, M. E., Y. V. Mailapur, S. K. Pradhan, G. A. Jacoby, P. Adams, C. L. Emery, P. L. May, and P. G. Pitsakis.** 2002. Regional occurrence of plasmid-mediated SHV-7, an extended-spectrum beta-lactamase, in *Enterobacter cloacae* in Philadelphia teaching hospitals. Clin. Infect. Dis. **35:**1551–1554.
14. **Munday, C. J., G. M. Whitehead, N. J. Todd, M. Campbell, and P. M. Hawkey.** 2004. Predominance and genetic diversity of community- and hospital-acquired CTX-M extended-spectrum beta-lactamases in York, UK. J. Antimicrob. Chemother. **54:**628–633.
15. **National Nosocomial Infections Surveillance System.** 2004. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1992 through June 2004, issued October 2004. Am. J. Infect. Control **32:**470–485.
16. **Pai, H., J. Y. Hong, J.-H. Byeon, Y.-K. Kim, and H.-J. Lee.** 2004. High prevalence of extended-spectrum β-lactamase-producing strains among blood isolates of *Enterobacter* spp. collected in a tertiary hospital during an 8-year period and their antimicrobial susceptibility patterns. Antimicrob. Agents Chemother. **48:**3159–3161.
17. **Park, Y. J., S. Y. Park, E. J. Oh, J. J. Park, K. Y. Lee, G. J. Woo, and K. Lee.** 2005. Occurrence of extended-spectrum beta-lactamases among chromosomal AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* in Korea and investigation of screening criteria. Diagn. Microbiol. Infect. Dis. **51:**265–269.
18. **Paterson, D. L., W. C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. McCormack, and V. L. Yu.** 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol. **39:**2206–2212.

19. **Paterson, D. L., L. B. Rice, and R. A. Bonomo.** 2001. Rapid method of extraction and analysis of extended-spectrum beta-lactamases from clinical strains of *Klebsiella pneumoniae.* Clin. Microbiol. Infect. **7:**709–711.
20. **Rhomberg, P. R., R. N. Jones, H. S. Sader, et al.** 2004. Results from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme: report of the 2001 data from 15 United States medical centres. Int. J. Antimicrob. Agents **23:**52–59.
21. **Rice, L. B., S. H. Willey, G. A. Papanicolaou, A. A. Medeiros, G. M. Eliopoulos, R. C. Moellering, Jr., and G. A. Jacoby.** 1990. Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-care facility. Antimicrob. Agents Chemother. **34:**2193–2199.
22. **Sanders, C. C., W. E. Sanders, Jr., and R. V. Goering.** 1982. In vitro antagonism of beta-lactam antibiotics by cefoxitin. Antimicrob. Agents Chemother. **21:**968–975.
23. **Sanders, W. E., Jr., J. H. Tenney, and R. E. Kessler.** 1996. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. Clin. Infect. Dis. **23:**454–461.
24. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
25. **Tsuji, A., A. Maniatis, M. A. Bertram, and L. S. Young.** 1985. In vitro activity of BMY-28142 in comparison with those of other beta-lactam antimicrobial agents. Antimicrob. Agents Chemother. **27:**515–519.
26. **Vuye, A., and J. Pijck.** 1985. In vitro antibacterial activity of BMY-28142, a new extended-spectrum cephalosporin. Antimicrob. Agents Chemother. **27:** 574–577.
27. **Zanetti, G., F. Bally, G. Greub, J. Garbino, T. Kinge, D. Lew, J.-A. Romand, J. Bille, D. Aymon, L. Stratchounski, L. Krawczyk, E. Rubinstein, M.-D. Schaller, R. Chiolero, M.-P. Glauser, A. Cometta, and the Cefepime Study Group.** 2003. Cefepime versus imipenem-cilastatin for treatment of nosocomial pneumonia in intensive care unit patients: a multicenter, evaluator-blind, prospective, randomized study. Antimicrob. Agents Chemother. **47:** 3442–3447.